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Variability in growth responses of non-O157 EHEC isolates in leafy vegetables, sprouted seed and soil extracts occurs at the isolate level.

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Abstract

Foods of plant origin are recognised as a major source of food-borne pathogens, in particular for Shigatoxigenic *Escherichia coli* (STEC). Most work for STEC and plant-based fresh produce has focused on the most prevalent outbreak serogroup, O157. However, non-O157 STEC are an emerging hazard, and as such it is important to characterise aspects within this group that reflect their ability to colonise alternative hosts and habitats relevant to horticultural production. Growth kinetics were quantified for a diverse set of clinical enterohaemorrhagic *E. coli* isolates in extracts made from different tissues of spinach, lettuce or sprouted seeds, or from soil, to represent association with ready-to-eat fresh produce production. For leafy vegetables, spinach apoplast supported the fastest rates of growth and lettuce root extracts generated the slowest growth rates. Growth rates were

similar for the majority of isolates in fenugreek or alfalfa sprouted seed extracts. Monosaccharides were the major driver of bacterial growth. No correlations were found for growth rates between different serotypes or for Shigatoxin gene carriage. Thus, growth rates varied in a plant-dependent and isolate-dependent manner, for all plant or soil extracts tested, indicative of isolate-specific differences in metabolic flexibility. These findings are relevant for risk assessment of non-O157 STEC.

Introduction

Fresh produce is a major vehicle of transmission of Shigatoxigenic *Escherichia coli* STEC, where foods of plant origin account for the majority of *E. coli* and *Shigella* outbreaks in the USA (Painter 2013) and are second only to beef/other meat globally (Hoffmann 2017) . Fresh produce is often eaten raw or minimally processed and leafy vegetables such as spinach and sprouted seeds have accounted for major outbreaks (Jay 2007, Buchholz 2011). STEC can use plants as secondary hosts (Holden 2015), which has implications for assessing the risk of infection from pre-harvest or post-harvest contaminated produce (Koseki, Isobe 2005, Huang 2012, Perez-Rodriguez 2014, Jensen 2015).

The ability of bacteria to grow in media containing plant tissue is important for assessing risk, and proliferation is influenced by a number of physio-chemico factors (Buchanan, Klawitter 1992). STEC growth capability on plant hosts is governed by several factors, including growth kinetics, biofilm formation and interaction with the plant defence response (Holden 2009). Our recent work showed a correlation between maximum growth rates in sprouted seed extracts and growth on sprouts for two STEC O157:H7 isolates, but not for leafy vegetables spinach and lettuce, where the plant tissue type had a major impact on growth rates and biofilm formation (Merget 2019). Risk assessments for STEC on fresh produce have been carried out, but the impact of plant tissue type is not normally included (Franz 2010, Danyluk, Schaffner 2011, Pang 2017).

The *E. coli* sub-species group of STEC comprise a diversity of genotypes and pathotypes, characterised by the presence of one or more bacteriophage that encode Stx toxin genes (Vila 2016). Recent work has shown that the presence of the Stx phage imposes a growth burden under certain conditions and transient metabolic rewiring. This occurred from introduction of two different Stx2a phages, φO104 from a O104:H4 serogroup strain and φPA8 from a O157:H7 serotype strain, into the *E. coli* K-12 laboratory strain MG1655 (Berger 2019). Although non-O157 isolates are often associated with fresh produce (Feng), e.g. O104 with fenugreek sprouts (Buchholz 2011), less is known about their ecology outside human hosts and how they may adapt their metabolism, than their O157 counterparts (Valilis 2018). This raises the question about capabilities of non-O157 STEC isolates to use environmentally derived substrates and proliferate in different hosts and habitats, as well as any influence or burden from carriage of the Stx bacteriophage. Therefore, we aimed to determine growth kinetics from a set of 12 non-O157 clinical enterohaemorrhagic *E. coli* (EHEC) isolates in the context of plant extracts from leafy vegetables (lettuce and spinach) and sprouted seeds (fenugreek and alfalfa sprouts), and soil extracts, to represent metabolism in a range of plant hosts and soil habitats, respectively. The EHEC group were selected on the basis of sequence types (ST) and included representative Stx positive and Stx negative isolates and an O104:H4 serotype Stx negative representative, to allow comparisons between different STs and between Stx phage carriage. Utilisation of clinical isolates generates relevant data, with added value over model or reference isolates that may have become lab-adapted.

Materials and Methods

Bacteria and defined media

The term EHEC refers to clinical isolates, while STEC includes all isolates that encode *stx* genes. The 12 clinical EHEC isolates (Table 1) were handled under BSL3 conditions throughout. Bacteria were cultured overnight in Lysogeny-broth medium (LB) at 37 °C (Bertani 2004), with shaking at 200 rpm. The overnight culture was inoculated 1:100 in 25 ml

rich defined 3-(N-morpholino)propanesulfonic acid (MOPS) medium (Neidhardt 1974) with 0.2 % glycerol and essential and non-essential amino acids, termed 'rich defined MOPS glycerol' (RDMG), for 24 h at 25 °C and 200 rpm. Bacteria were adjusted to the required starting optical density (OD) 600 nm prior to experiments. Defined artificial 'lettuce apoplast' or 'sprout extract' media was generated by adding each group of constituents to a base minimal MOPs medium (MMM) without carbon source and amino acids, exactly as described previously (Merget 2019), reproduced in Supplementary Table 3. Three variants were made each with reduced concentrations of each component group, by dilution of one major group at a time: 1:50 monosaccharides (MS), 1:10 amino acids (AA) or 1:20 organic acids (OA), while the other groups were at 1:1. Viable counts were determined by diluting samples in PBS, plating the dilutions on MacConkey (MAC) agar, incubation overnight at 37 °C and counted manually the next day. The experiments were conducted in triplicate. Viable counts and OD_{600} nm were plotted in Graphpad Prism (v8).

Plant and soil extracts

Leaf lysate, root lysate and apoplastic washings were generated from lettuce (*Lactuca sativa*) var. All Year Round and spinach (*Spinacia oleracea*) var. Amazon, while whole sprout lysates were generated from fenugreek (*Trigonella foenum-graecum*) and alfalfa (*Medicago sativa*). All plants were propagated and extracts made exactly as described previously (Merget 2019). Soil extract medium (SEM) was produced as described previously (Brennan 2010) from a Scottish soil sourced from a James Hutton Institute mixed arable and livestock farm, and subject to elemental analysis (gley soil type: organic C = 10.48 %, N = 0.70 %; Supplementary Table 1 for inorganic analysis). A slurry was made at a soil:buffer ratio of 4:9 with McIlvaine's medium (18 mM citric acid; 5 mM K_2HPO_4; 0.4 mM $MgSO_4*7H_2O$; 56.6 mM Na_2HPO_4; 7.6 mM $(NH_4)2SO_4$; 3 µM thiamine, 6 µM $(NH_4)2SO_4*FeSO_4*6H_2O$; 0.4 % (w/v) glycerol), manually shaken by inverting for 5 min, autoclaved for 1 h at 121°C, and allowed to settle overnight. The supernatant was then removed, centrifuged at 5,000 x g for 15 min, re-autoclaved for 20 min at 121°C.

Bacterial growth rates

Growth dynamics were assessed at 25 °C, which is relevant to plant growth in the Northern temperature zone, using a high-throughput plate reader. The *E. coli* isolates were grown as described above, adjusted to an OD_{600} of 0.05 in PBS (~ 2.1 x 10^7 cfu ml^{-1}) and inoculated at a 1:10 dilution (i.e. 20 µl) in plant or soil extracts (at 1:20 w/v in dH_2O) or defined media (RDMG), in 200 µl total volume, in multi-well plates. The EHEC isolates were grown at 25 °C in a pre-warmed Infinite F200 Pro plate reader (Tecan, Switzerland) using 96-well multi-well plates. Samples were pipetted in duplicate in a randomised manner on the plate, with negative controls included. All growth curves in extracts were repeated independently three times (i.e. n = 6). Measurements (at 595 nm) were recorded every 15 min for 48 hours and multi-well plates were shaken for 60 seconds pre- and post-measurement. Results were exported from plate reader proprietary software as tab-delimited files. For model fitting, the replicates of each isolate and medium type were averaged (outliers that did not have the same growth dynamics were removed) and converted to viable counts log (cfu h^{-1}), using a conversion factor of 4.2 x 10^8 cfu ml^{-1} and all growth curves were modelled using the DM-Fit add-in in MS Excel, as described previously (Merget 2019). A non-linear Baranyi model successfully fitted 96 % of the growth curves to an R^2 value of at least 0.9 (Supplementary Table 2). Statistical analysis (ANOVA, correlations) were carried out in GraphPad Prism (v8) and VSNI Genstat (v19) programs.

Results

E. coli growth rates in plant and soil extracts

The EHEC isolate set comprises four pairs of the same sequence types (ST) in serogroup O26, O103, O121, O145 with Stx positive and Stx negative representatives; a pair in serogroup O26 with Stx1 and Stx1+2 representatives; a Stx negative representative of the O104:H4 serotype; and an additional O145:NM serotype Stx negative representative (Table

1). Growth dynamics of the EHEC isolates (Supplementary Table 2) were measured in the plant extracts from representative edible species: two leafy greens (lettuce, spinach) and two sprouted seeds (fenugreek, alfalfa) (summarised in Table 2). Plant tissues for the leafy greens of total lysates of leaves, total lysates of roots, and apoplastic washing recovered from leaves represented edible, non-edible and internalised tissues, respectively.

In general, EHEC growth rates in the leafy vegetable extracts produced similar patterns between the isolates, but this was not as obvious in the sprouted seed extract (Fig. 1). Similar patterns of EHEC growth rates occurred in spinach and lettuce extract types, whereby apoplastic extracts supported the fastest growth rates while roots extracts generated the slowest growth rates. The highest growth rates occurred in spinach extracts compared to lettuce or the sprouted seeds (Fig. 1A 'vs' Fig. 1B, IC), with a significant difference between plant species types ($p < 0.001$: two-way ANOVA on isolates & all plant species). Differences between growth rates in the sprouted seed extracts had a higher dependence on the isolate than the extract / plant species type (Fig. 1C), with 8.80 % and 4.47 % of variation from 'isolates' and 'species', respectively; $p < 0.0001$ for isolate, species or interaction (two-way ANOVA on and fenugreek/alfalfa). Growth rates in the no-extract control medium (RDMG) was relatively consistent between the isolates, ranging between 0.069 – 0.112 cfu h^{-1} (Fig. 1D) and supported the highest growth maxima of the conditions tested, i.e. the upper asymptote of the sigmoid curve. The isolates exhibiting the highest growth rates were EHEC isolate 3905 (O145:NM Stx+) in spinach and lettuce extracts, while EHEC isolate 3907 (O145:NM Stx-) grew fastest in fenugreek spout extracts. The lowest growth rates were observed in lettuce root lysate from EHEC isolates 3905, 3917 (O121:H19 Stx-) and 3900 (O26:NM Stx+).

Growth dynamics were measured in soil extracts to mimic growth in soil and soil contaminated irrigation water. In general, the maximum growth rates of the clinical EHEC isolates was lower than that seen in the no-extract control medium (RDMG), but not universally (Fig. 1D). The highest growth rate was seen for isolate 3916 (O121:H19 Stx+)

and the lowest for isolate 3900 (O26:NM Stx+). The degree of variance between isolates (18.42 %) was similar to that for soil extract and RDMG media types (16.73 %: two-way ANOVA on isolates and SEM/RDMG). Growth maxima was significantly restricted in soil extracts (Supplementary Table 2) to an average of 8.41 log cfu (SD 0.08) compared to 9.05 log cfu (SD 0.04) in RDMG for all isolates grouped together ($p < 0.0001$: one-way ANOVA on 'media type').

Comparison of the growth rates of the EHEC clinical isolates in plant extracts with that of a set of reference *E. coli* isolates, including two in serogroup O157, measured in a previous study (Merget 2019), showed significantly lower growth rates for the EHEC clinical isolates ($p < 0.0001$: two-way ANOVA on 'isolate' and 'media type' factors). Comparisons between independent experimental set-ups were made possible from data normalised to cfu h^{-1} and a reference isolate (O157:H7 isolate Sakai) that was measured in both systems in no-extract medium (RDMG). There was no significant difference in μ for the reference (Sakai) between either of the plate readers (Infinite F200 Pro, 0.098 ± 0.0024 log (cfu h^{-1}) used here; Bioscreen C, 0.097 ± 0.0019 log (cfu h^{-1}) used previously), showing that the difference with the EHEC clinical isolates was real.

The influence of plant extract metabolites on *E. coli* growth

To determine any influence of the major metabolite groups on growth rates of the isolates in the plant extracts, defined, 'artificial' growth media were generated that mimicked the main plant extract metabolites. The six principal metabolites were selected from lettuce apoplast or sprout extracts to represent contrasting metabolite profiles (Supplementary Table 3, taken from (Merget 2019)). The impact of the major groups of monosaccharides (MS), organic acids (OA) or amino acids (AA) were assessed separately from dilutions, to restrict their effect, i.e. 1:50 MS, 1:10 AA or 1:20 OA, while the other groups were at 1:1. Higher growth rates occurred in the sprout extract artificial medium compared to lettuce artificial medium ($p < 0.0001$: t-test on undiluted lettuce and sprout artificial media), and the growth rate patterns of the isolates were similar in both media types (Fig. 2). Restriction of amino acids or organic

acids in lettuce medium, or amino acids in sprouted seed medium had minimal or no impact on maximum growth rates, while restriction of the monosaccharide group (MS) resulted in ~ 3-fold decrease in growth rate under all conditions tested (Fig. 2). The MS-dependent effect occurred for all EHEC isolates (39.04 % variation), although there were also significant isolate dependencies (4.75 % variation, $p < 0.0001$: two-way ANOVA on 'isolate' and all iterations of lettuce/sprout artificial media type).

Influence of Stx phage on growth rates

To determine the influence of the Stx phage on growth rates, ANOVA was carried out for the set of EHEC isolates grouped by Stx phage carriage (presence or absence). There was no significant difference in growth rates for the extracts except of lettuce root lysate, where the average growth rate was lower for isolates encoding Stx phage compared to those without Stx (0.0132 log cfu 'vs' 0.0231 log cfu: p 0.041; one-way ANOVA on 'Stx carriage'). There was no difference in growth rates between Stx carriage for the artificial media types. Neither was there any difference in growth rates by serotype, for all of the media types tested. Therefore, difference in EHEC maximal growth rates did not vary on a toxin- or serotype-group basis, rather on an individual isolate basis.

Discussion

The most common STEC associated with food-borne illness belong to serogroup O157, which has become the archetypal serogroup for research, yet the STEC sub-species includes a diversity of genotypes with an estimate 470 serotypes (FAO/WHO STEC Expert Group 2019). Non-O157 STEC serogroups have been associated with food-borne illness from plant-derived foods (Feng), including the very large-scale outbreak of serotype O104:H4 from fenugreek sprouts in 2011 (Buchholz 2011), and serogroup O26 associated more unusual vehicles such as flour (CDC 2019) (H-type not provided). Since the number of reported EHEC outbreaks from non-O157 STEC appears to be increasing (Valilis 2018), and foods of plant origin represent a major source of food-borne transmission (Hoffmann 2017)

there is a requirement to investigate how non-O157 STEC are able to persist in alternative hosts and habitats in order to generate more robust risk assessments.

Plant extracts are representative of damaged or injured plant tissue, which could occur pre-harvest or during production and packaging, and growth rates in these tissues can be used to inform worst-case scenarios, like failures in critical control points (e.g. refrigeration). Equally, soil extracts are representative of soil-contaminated irrigation water and direct contamination of soil. Our previous work showed a correlation between the growth rates of O157:H7 STEC isolates in extracts from sprouted seeds (alfalfa or fenugreek) and growth on the growing sprouts (Merget 2019), but not for leafy vegetables (lettuce or spinach) as growth rates were restricted in the living plants. This indicated specificity in the interactions, and influence from the plant defence response. Here, a large degree of variation occurred between non-O157 clinical EHEC isolates in their capacity to grow in plant and soil extracts, with no apparent association with serotype or Stx carriage.

Apoplast extracts, representative of the internal environment of leaves, tended to support the highest growth rates of the isolates, especially from spinach. This mirrored the situation for STEC serotype O157:H7 growth rates in spinach tissue, where the bacteria also exhibited an ability to internalise into leaves and roots of living spinach plants (Merget 2019). In contrast, the lowest growth rates tended to occur in root extracts, especially for lettuce. Edible lettuce is well-known for phenolic secondary metabolites, which are bitter-tasting and inhibitory to bacterial growth (Daglia 2012). Less is reported on the root tissue metabolite composition, but inhibitory intracellular compounds may have been released in the generation of the whole root lysate extracts, since in contrast, the rhizosphere of living lettuce plants enable STEC serotype O157:H7 to persist for at least 10 days (Wright 2017). Growth rates in sprouted seeds extracts varied between the isolates, and for most (9/12) there was little difference based on plant species (fenugreek ‘vs’ alfalfa), but for three isolates (in serogroup O145, O121) there were more marked differences, indicative of a degree of specificity.

In general, the patterns of growth kinetics of the non-O157 panel was similar to the O157 isolates tested previously (Merget 2019), although the growth rates tended to be lower. The O157 isolates tested may have a comparatively enhanced metabolic capacity for plant-derived substrates since they were from plant-associated outbreaks (white radish sprout; lettuce). The clinical *E. coli* O104:H4 isolate tested here (Zangari 2013) was collected in the same 2011 outbreak context as the isolate from fenugreek seeds (Buchholz 2011) and although a level of adaptation to fenugreek sprouts has been suggested for the O104:H4 isolate (Juneja 2014, Grad 2012), there is also evidence to show that other serotypes (e.g. O157:H7) are more capable on plants, in terms of seed survival (Knodler 2016) and growth on sprouted seeds (Xiao 2014, Knodler 2016).

Plant and soil extracts contain complex mixtures of metabolites, and it is most likely that differences in metabolic capacity between the isolates explain the growth rate differences. This is supported by metabolic profiling of different STEC serotypes, which showed distinct clusters based on carbohydrate fermentation (Posse 2007), and restrictions in some metabolic pathways. Serogroup O26 were unable to ferment l-rhamnose, which is a major component of plant cell wall pectin, complexed as rhamnogalacturonan (Caffall, Mohnen 2009). Similarly, some serogroup O103 and O26 isolates could not ferment l-arabinose, also a component of pectin, which could partly explain the lower growth rates of these isolates on lettuce root exudates, since pectin can be present in root mucilage (Caffall, Mohnen 2009). Freely available arabinose was detectable in all of the plant extracts, but at minimal levels compared to the major glycans of sucrose, glucose and fructose (Merget 2019). One of the most abundant glycans in lettuce root lysates is raffinose (Merget 2019), which was not fermented by the O145 serotypes (Posse 2007). However, the three O145 isolates tested here were not restricted for growth, with the exception of isolate 3905 in lettuce root lysates, indicative of redundancy in the inability to ferment raffinose. Some isolates of serogroup O26 were restricted for growth in soil extracts, suggesting a common genetic component to metabolic pathway limitation. It was notable that the growth maxima was universally lower in

soil extracts than in RDMG or the sprouted seed extracts, implying earlier exhaustion of a key metabolite(s). Growth rates in the artificial media were only reduced when the monosaccharides were limited, showing a dominant effect over organic or amino acids, as for STEC O157 (Merget 2019). This suggests that the soil extracts were lacking in a saccharide component that reduced growth maxima.

Carriage of the Stx bacteriophages did not show any impact on growth rates of the isolates under the conditions tested; although there was a reduction for the Stx-positive group in lettuce root lysates, growth rates in this media type were relatively low compared to other substrates. This contrasts with the restriction in growth rates observed following introduction of Stx2a into a non-STEC genetic background (Berger 2019). The difference is likely due to the complex complement of metabolites in the extracts compared to a minimal medium supplemented with a single metabolite, thereby resulting in redundancy in the response. Indeed, this is supported by the growth rates in diluted artificial media, where there is one example of enhanced growth rates for the Stx+ isolate (3903) compared to its Stx- counterpart (3904), in either lettuce or sprout artificial media restricted in monosaccharides. Therefore, carriage of the Stx phage did not appear to impose any disadvantage in growth in either plant extracts or soil extracts, for the non-O157 serogroups tested.

STEC is classified for risk to human health on the basis of virulence gene carriage (FAO/WHO STEC Expert Group 2019), but serotype classification is useful for historical context. The group of isolates tested here represent of a diverse group of isolates associated with clinical enterohaemorrhagic disease, although the source attribution is unknown (Bielaszewska 2008), and could be meat / vegetable food or environmental. Here, we found that neither serotype nor carriage of the Stx virulence factor influenced their growth in soil or plant extracts, which was dependent instead on the specific isolate type and extract type. This implies that it is the metabolic flexibility of *E. coli* that allows certain isolates to inhabit alternative hosts and habitats.

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Conflict of interest disclosure

The authors declare no conflicts of interest.

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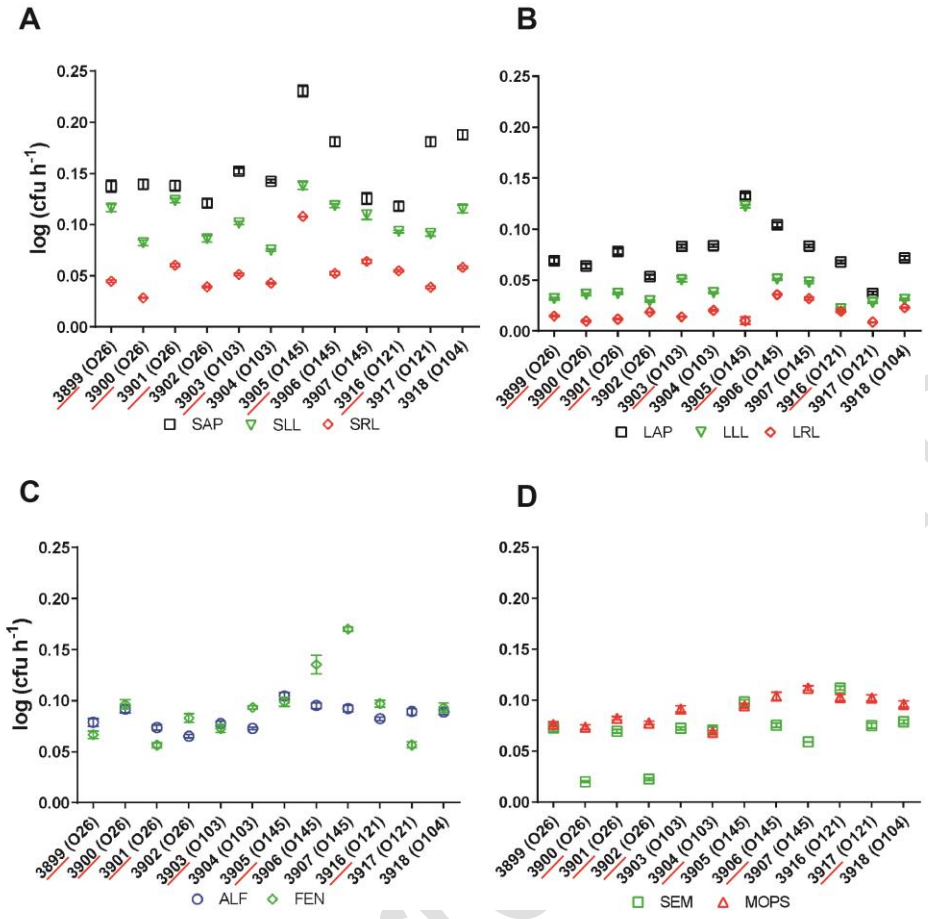

Figure 1 Maximum growth rates (μ) of clinical EHEC isolates in plant extracts.

Maximum growths rates (μ) expressed as log (cfu hr^{-1}) calculated using the Baranyi model for the EHEC isolates in in spinach **(A)** or lettuce **(B)** extracts: aploplast (black squares), leaf lysates (green triangles) and root lysates (red diamonds) extracts; or in alfalfa (green diamonds) or fenugreek (red circles) sprouts lysate extracts **(C)**; or in soil extract medium (SEM) (green squares) or in RDMG (red triangles) as no-plant extract control **(D)**, at 25 °C. Individual isolate names are provided along with the relevant serogroup (O26; O103; O145; O121; O104) and red underlines indicate Stx+. Each point is the average rate (n = 6), with standard errors of the model fit indicated by bars.

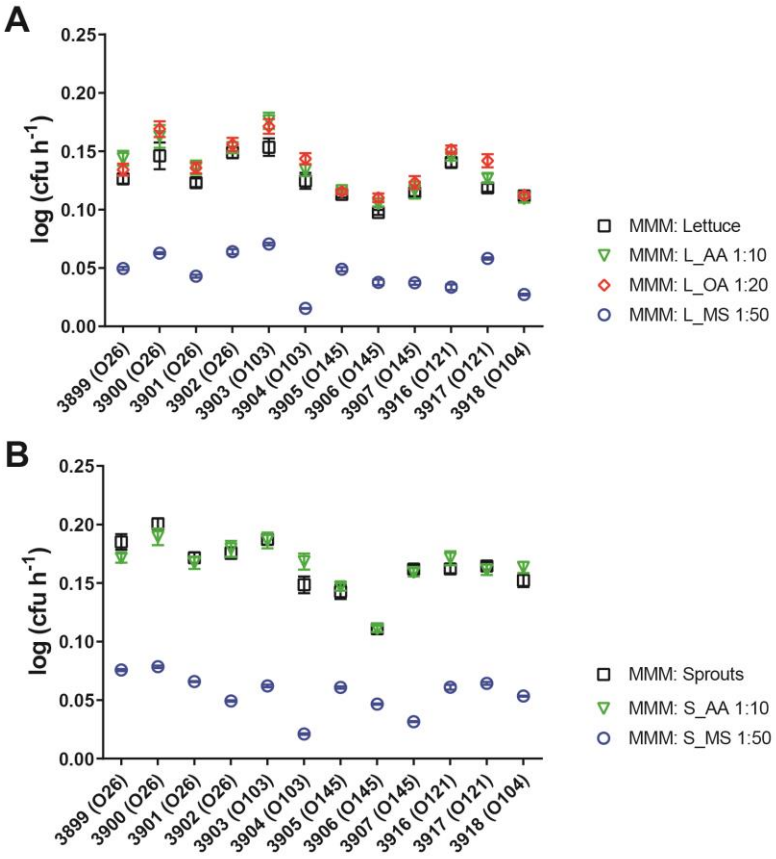

Figure 2 Maximum growth rates (μ) in artificial media mimicking plant extracts.

Maximum growths rates (μ) expressed as log (cfu hr^{-1}) calculated using the Baranyi model for the EHEC isolates at 25 °C in artificial media mimicking (**A**) lettuce apoplast or (**B**) sprout lysates (a mixture of alfalfa and fenugreek sprout metabolites) at specified dilutions. The base minimal MOPS medium (MMM) was supplemented with monosaccharides (MS), organic acids (OA) or amino acids (AA) for lettuce (L) or sprout (S) media at the dilution specified (AA 1:10; OA 1:20; MS 1:50), or all three component undiluted (MMM:Lettuce; MMM:Sprouts). Individual isolate names are provided along with the relevant serogroup (O26; O103; O145; O121; O104) and red underlines indicate Stx+. Each point is the average rate with standard errors of the model fit indicated by bars.

Table 1 Clinical EHEC and EPEC isolates used in this study

EHEC clinical isolates were used with permission from Prof. Dobrindt (University of Münster). ST = sequence type, Stx = Shiga toxin designation, FliC = flagella types (N.B. non-motile isolates still encode flagella), Patho = pathotype.

Isolate Name	**Serotype**	**FliC**	**ST**	**Stx**	**Patho**	**Reference**
3899 (2245/98)	O26:H11	H11	21	Stx1	EHEC	(Mellmann 2008)
3900 (5080/97)	O26:NM	H11	21	Stx1+Stx2	EHEC	(Mellmann 2008)
3901 (1530/99)	O26:H11	H11	29	Stx2	EHEC	(Mellmann 2008)
3902 (1676/99)	O26:H11	H11	29	negative	EPEC	(Bielaszewska 2008)
3903 (2969/99)	O103:H2	H2	17	Stx2	EHEC	(Unkmeir, Schmidt 2000)
3904 (4931/00)	O103:H2	H2	17	negative	EPEC	(Bielaszewska 2008)
3905 (5122/99)	O145:NM	H28	32	Stx2	EHEC	(Bielaszewska 2008)
3906 (6519/95)	O145:NM	H28	32	negative	EPEC	(Bielaszewska 2008)
3907 (488/99)	O145:NM	H28	32	negative	EPEC	(Bielaszewska 2008)
3916 (2763/99)	O121:H19	H19	655	Stx2	EHEC	(Bielaszewska 2008)
3917 (6316/94)	O121:H19	H19	655	negative	EPEC	(Bielaszewska 2008)
3918 (C227/11ϕcu)	O104:H4	H4	678	negative	EPEC	(Zangari 2013)

Table 2 Growth media used in the study

Name	Components	Use
RDMG	Rich defined MOPS glycerol	Control medium for inoculation of bacteria into plant and soil extracts
Leaf apoplast	Apoplast washings from spinach or lettuce leaves	Used neat
Leaf lysate	A whole cell lysate of spinach or lettuce leaves	Used neat
Root lysate	A whole cell lysate of spinach or lettuce roots	Used neat
Sprout lysates	A whole cell lysate of fenugreek or alfalfa sprouts	Used neat
Soil extract	A McIlvaine's medium-extract of Scottish soil	Used neat
MMM	Minimal MOPS medium	Base medium for generating artifical lettuce and sprout medium
AA	Amino acid mixture derived from lettuce or sprouts	used to supplement MMM at neat or 1:10 dilution
OA	Organic acid mixture derived from lettuce or sprouts	used to supplement MMM at neat or 1:20 dilution
MS	Monosaccharide mixture derived from lettuce or sprouts	used to supplement MMM at neat or 1:50 dilution